PROGRAMME CRYOCONSERVATION OF LYMPHOCYTES IN THE PRACTICE OF IMMUNOLOGICAL DEPARTMENT OF TRANSPLANTATION CENTER

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Recently in all immunological laboratories of world transplantation centers is applied in larger scales the method of cryoconservation of human lymphocytes (Strong, 1975). One of most important clinical-practical directions for applying lymphocyte cryoconcervation is determination of the level and character of recipients' sensibilization until and after transplantation by using "panell" cell-test of known HL-A phenotype. The method of investigation of pre-existing lymphocytotoxical antibodies towards antigens of the resological sublocuses HL-A (A, B, C) has been applied as a routine one in the clinical practice of the Institute of Transplantation and Artificial Organs (Moscow, USSR) by Zaretskaya Y. M. since 1978. Until now in the laboratory of Immunogenetics a number of over 800 sera of recipients waiting kidney grafts has been tested. However, certain disadvantages of the cited method is the considerable decrease of vitality of the cells after a short period of preservation at —80° C (3—3.5 months). From the other hand, the undoubtful role of DR-serology for clinical transplantation and suitable selection of donor-recipient, as well as the determination of the recipient's immunological status in pre- and post-transplantation period in the scales of immunological monitoring, proves the necessity of new methods for longer preservation and conservation of lymphocytes and donor's B-cells.

We studied the vitality of lymphocytes from peripheral blood and B-cells set to a programme freezing and kept in a liquid nitrogen for a period of two months; we also investigated the influence of the cryoconservation upon those structures on lymphocytes' cell membranes which determine the system antigen histocompatibility, serologically studied (sublocuses HL-A: A, B, C).

Our study covers cells separated from blood of healthy volunteers. The lymphocytes were isolated out of peripheral blood by using the method of Böym (1969) in the density-gradient of verographin-ficol. B-cells were separated by columns with a synthetic cotton after El-Awar (1980). Lymphocytes in medium 199 were mixed with 20% inactivated AB-serum (IV) and just before freezing was added a 10% solution of DMSO. All stages of mixing were performed on ice surface. The following cell concentrations were investigated: 2.5×10^4, 5.0×10^4, 2.5×10^5, 5.0×10^5, 2.5×10^6, 5.0×10^6. The freezing itself was done in the apparatus for cryoconservation PLANER with an initial speed of 1° C per minute until —50° C and further more with speed of 15° C per minute until —196° C. After that the test-tubes with lymphocyte suspension were placed in a liquid nitrogen. Melting of the cells was done at 37° C for 1 minute.

We studied the influence of cell washing with DMSO upon vitality and immunological activity of lymphocytes. For that reason part of the melted suspension was washed out from the cryoprotector by using the method of

a degree-6-times dilution adding drop by drop to the volume of the suspension 5 equal parts of 20% glucose solution in Henks' solution together with 1 same volume of inactivated AB serum (IV). The exposure of each portion was 2 minutes. To determine the vitality of the cells we incubated them for 25 minutes in 0.3% solution of Trypan-blue and later counted the vital cells in Goryaev's chamber. The lymphocytotoxical test for determination of HL-A phenotype was performed after the standard variant of Kissmeyer-Nielsen-Thorsby (1970).

The results are presented on tables 1 and 2. It is obvious that the more the cell concentration increases, less vital the lymphocytes are. Maximum

Table 1

Vitality of cryoconserved lymphocytes with various periods of preservation (M±m)

Concentration of lympho-cytes/ml	Lower vitality of lymphocytes after cryoconservation and preservation in liquid nitrogen (%) for various periods					
	After one week		After one month		After two months	
	without washing	with washing	without washing	with washing	without washing	with washing
			PBL			
2.5×10^4	1.0±0.58	0.67±0.67	0.33±0.33	1.67±0.34	1.0±1.0	1.0±0
5.0×10^4	0	1.0 ±0.11	10 ±0.58	1.0 ±0.58	0.5±0.5	1.0±1.0
2.5×10^5	2.5±0.77	1.33±0.77	1.33±0.77	2.0 ±0.58	1.0±0	2.5±0.5
5.0×10^5	1.0±0.41	4.0 ±0.41	3.0 ±0.58	2.67±0.34	3.5±0.5	4.0±1.0
2.5×10^6	0	1.0 ±1.0	1.0 ±1.0	1.5 ±1.5	2.0±1.0	2.0±2.0
5.0×10^6	1.75±1.75	3.0 ±1.0	3.0 ±1.0	2.0 ±1.15	3.5±1.5	3.0± 0
			B-cells			
2.0×10^4	0	0	0	0	0	0
4.0×10^4	0	0	0.5±0.5	0	—	—
2.0×10^5	2.0 ±0.5	1.0±0.33	3.0±0.67	0	3.0±0.5	2.0±0.33
4.0×10^5	2.67±1.22	2.5±0.87	0.5±0.5	1.33±0.88	—	—
2.0×10^6	6.5 ±0.5	6.0±1.0	7.0 ±0	5.5 ±2.5	5.0±1.0	6.5±0.5

Table 2

Influence of the lymphocyte cryoconservation upon the results of the manifestation of antigens HLA-A and HLA-B

Number of donor	Vitality			Phenotype		
	until the cryoconservation	1 month later	2 months later	until the cryoconservation	1 month later	2 months later
1	100	96	96	A 9.10 Bw16(38—39)	A 9.10 Bw16(38—39)	A 9.10 Bw16(38—39)
2	99	95	95	A 2.3 B 14.27	A 2.3 B 14.27	A 2.3 B 14.27
3	100	96	96	A 1.2 B 17.40	A 1.2 B 17.40	A 1.2 B 17.40
4	100	96	96	A 2.28 B 18 39	A 2.28 B 18.39	A 2.28 B 18.39
5	99	96	96	A 3.19 B 7.40	A 3.19 B 7.40	A 3.19 B 7.40

vitality decrease after a 2-month preservation in liquid nitrogen is 4% for the lymphocytes and 7% for B-cells; it is absolutely acceptable for their application in the lymphocytotoxicity test. Statistical difference in the lymphocytes' vitality with or without washing out of the cryoprotector was not established. It was also found out that the cryoconservation after the programme PLANER and preservation of the cells in liquid nitrogen for a period of 2 months did not change the character of manifestation of transplantation antigens in serological sublocuses HL-A (A, B, C).

Our further aim is to continue the study of vitality of lymphocytes and B-cells and their immunological preservation together with some actual cell tests (MLC, CML) by setting them to a cryoconservation programme and long preservation in liquid nitrogen for a period of 2 years.

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ПРОГРАММНОЕ КРИОКОНСЕРВИРОВАНИЕ ЛИМФОЦИТОВ В ПРАКТИКЕ ИММУНОЛОГИЧЕСКОЙ СЛУЖБЫ ТРАНСПЛАНТАЦИОННОГО ЦЕНТРА

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РЕЗЮМЕ

Проведено всестороннее изучение вопросов применения программного криоконсервирования лимфоцитов в практичекой работе трансплантационного центра. Изменение жизнеспособности лимфоцитов и их иммунологическая сохранность нами детально анализированы в целях их практического применения в клинике.